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Association of Nephrolithiasis and Gene for Glucose Transporter Type 9 (SLC2A9): Study of 145 Patients

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Aim To investigate the association of nephrolithiasis and solute carrier family 2, facilitated glucose transporter, member 9 (SLC2A9), also known as glucose transporter type 9, Glut9.

Methods A total of 145 participants were recruited in the period April-October 2008 from the Department of Mineral Research of the Medical School Osijek, Osijek, Croatia; 58 (40%) had confirmed nephrolithiasis and 87 (60%) were asymptomatic. Four single nucleotide polymorphisms (SNP) from the SLC2A9 gene were genotyped in both groups (rs733175, rs6449213, rs1014290, and rs737267).

Results There was a weak but significant association of all 4 SNPs and nephrolithiasis ($P=0.029$ for rs733175; $P=0.006$ for rs6449213; $P=0.020$ for rs1014290, and $P=0.011$ for rs737267). Logistic regression in an age- and sex-adjusted model suggested that genotype C/T for rs6449213 had odds ratio for nephrolithiasis of 2.89 (95% confidence interval 1.13-7.40). This SNP explained a total of 4.4% of nephrolithiasis variance.

Conclusion Development of nephrolithiasis may be associated with SLC2A9 gene. Further studies are needed to clarify the role of SLC2A9 gene as a link between uric acid and nephrolithiasis.

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Renal stone formation (nephrolithiasis) is a disease characterized by the existence of solid deposits in the upper parts of the urinary tract (1). It is estimated to affect between 3%-9% of the population, with large differences between various populations (2,3). There is a number of causes that may lead to the renal stones formation, including diet and obesity status, some drugs, other diseases, climate changes, metabolic disorders, and genetic factors (2,4,5). The complexity of this disease caused researchers to consider nephrolithiasis as one feature of a broader systemic disease, rather than a local disease restricted to a single organic system (6). This is especially interesting in relation to gout and metabolic syndrome, which are both systemic disorders in close relation to nephrolithiasis (6-8). Even the cohort studies have confirmed the association of gout and kidney stones, suggesting that the history of gout increases the risk for kidney stones (9). Another study showed that, in the age-adjusted model, gout had an odds ratio of 1.97 for previous kidney stones (95% confidence interval [CI], 1.37-2.83) and that even after adjustment for sex, race, body mass index, and presence of hypertension the odds ratio remained significant (10).

Genetic contribution to renal stones formation has been identified long time ago (2). In line with these suggestions, heritability of some of the traits associated with nephrolithiasis has been shown to be as high as 95% (11). Heritability of the urinary stones was reported to be lower (56%) (12), but still sufficiently high to be considered a substantial genetic proportion of variance and suggesting that it may be under genetic control. So far, a number of studies have established a link between predominantly oxalate kidney stones and several genes, including vitamin-D receptor gene (VDR) and calcitonin receptor (CTR) gene (13), heparan sulfate (HSPG2) gene (14), or fibronectin gene (FN1) (14).

The quantitative trait associated with nephrolithiasis is the serum uric acid concentration, which is under strong genetic control by the gene for glucose transporter type 9 (SLC2A9 or Glut 9) (15). The gene was initially described in an isolated island community (16,17), where genetic properties of the population are expected to act in favor of facilitated gene mapping efforts (18). Subsequent meta-analysis of 14 populations confirmed the association of this gene with serum uric acid concentrations (19). This led to a number of clinical studies that have confirmed its involvement in the uric acid metabolism, including urate handling in the kidney and uptake in the liver (20,21). Based on the previous suggestions that gout and nephrolithiasis may share a common pathway (15), it might be interesting to see if SLC2A9 could explain the commonalities in patients with any of the following conditions. Therefore, the aim of this study was to investigate the association of nephrolithiasis and genetic variants of the SLC2A9.

PARTICIPANTS AND METHODS

We investigated 145 patients from the Department of Mineral Research of the Medical School Osijek, Osijek, Croatia. The sampling was based on the hospital nephrolithiasis register with over 2000 entries since 1988, but only patients with confirmed urate nephrolithiasis were included in the sampling frame. After identification, a postal invitation was sent to all eligible patients (over a hundred of participants), 58 of which responded and were included in the study. The diagnosis of nephrolithiasis was based on the history of renal colic, with confirmed hematuria and voiding of the calculus or previous surgical and endoscopic removal of stone(s), or radiographic evidence of stone(s), and the report of stone analysis (3,5). The participants were invited and their samples were collected from April-October 2008. Further 87 patients who were undergoing osteoporosis diagnostics and had no recorded symptom associated with nephrolithiasis were recruited from the same department by the consecutive sampling in April-October 2008. The following participants were excluded: participants with gout, gastro-intestinal diseases (ulcerative colitis, malabsorption syndrome, chronic pancreatitis), renal tubular acidosis, pregnant women, participants over 75 years, participants with primary hyperparathyroidism and other diseases affecting calcium metabolism (hyperthyroidism, acromegaly, sarcoidosis, diabetes, or cancer), and participants using drugs such as corticosteroids, barbiturates, estrogen, or vitamin D. Written consent was obtained from all participants and the study was approved by the Ethics Committee of the Medical School Osijek, Osijek, Croatia.

We performed genotyping for both groups, including 4 single nucleotide polymorphisms (SNP) from the SLC2A9 gene: rs733175, rs6449213, rs1014290, and rs737267. The selection of these 4 SNPs was based on the results from a previous study, which suggested that these SNPs showed the strongest association with uric acid concentration (15). These SNPs have different locations within a gene: rs733175 is located at 5' end, rs6449213 within intron 4, rs1014290 within intron 3, and rs737267 within intron 7, all on the chromosome 4 within the region

4p16.1 (9,260-9960 kb) (15). All SNPs were genotyped using TaqMan SNP Genotyping assays and fluorescence was performed using an ABI 7000 sequence detection system (Applied Biosystems, Foster City, CA, USA). Genotyping procedures were performed according to the manufacturer's protocols, except for the PCR amplifications, which were carried out in half of the original volume, with all reagents volumes adjusted accordingly (22). Genotyping procedures were performed in the DNA laboratory of the J. J. Strossmayer University of Osijek, School of Medicine.

Statistical analysis

The data were analyzed as the proportions of the genotypes in each group. Due to the small number of participants, Fisher exact test was used, performed by the internet-based Simple Interactive Statistical Analysis (23), using the 2×5 Fisher exact test option. This is a generalization of the Fisher exact test, which calculates an exact probability value for the relationship between more variables, based on the two loops analysis, one for each degree of freedom (23). Additionally, descriptive statistics for numerical variables and the calculation of Mann-Whitney test and logistic regression were performed using R package (The R Project for Statistical Computing, version 2.10.1, Bell Laboratories, NJ, USA; available from http://www.r-project.org/). Level of significance was set at $P < 0.05$.

RESULTS

A total of 145 participants were included in this study: 58 (40%) had symptoms of nephrolithiasis and 87 (60%) had no symptoms of nephrolithiasis and were thus considered asymptomatic. The symptomatic participants were older, less likely to be women, and had significantly greater body mass index and waist circumference (Table 1).

The SNP analysis indicated significant differences among symptomatic and asymptomatic participants in all 4 SNPs, with the largest difference for rs6449213 (Table 2). Since the sample size was rather small for a detailed analysis, only this SNP was included in the logistic regression model, which was adjusted for the effects of age and sex. Odds ratio for being heterozygous at this SNP and developing nephrolithiasis was 2.89 (95% CI, 1.13-7.14) (Table 3). The entire model with all 3 variables explained a total of 31.2% of variance, while the rs6449213 explained 4.4% of total nephrolithiasis variance.

TABLE 2. Genotype frequencies (percentages) in symptomatic and asymptomatic participants included in the present study

Marker/ status	Symptomatic participants	Asymptomatic participants	Total	*P**
rs733175				
TT	30 (51.7)	50 (57.5)	80 (55.2)	0.029
CT	24 (41.4)	33 (37.9)	57 (39.3)	
CC	4 (6.9)	4 (4.6)	8 (5.5)	
rs6449213				
TT	30 (51.7)	57 (65.5)	87 (60.0)	0.006
CT	25 (43.1)	24 (27.6)	49 (33.8)	
CC	3 (5.2)	6 (6.9)	9 (6.2)	
rs1014290				
TT	27 (46.6)	47 (54.0)	74 (51.0)	0.020
CT	25 (43.1)	31 (35.6)	56 (38.6)	
CC	6 (10.3)	9 (10.3)	15 (10.3)	
rs737267				
GG	28 (48.3)	51 (58.6)	79 (54.5)	0.011
GT	26 (44.8)	28 (32.2)	54 (37.2)	
TT	4 (6.9)	8 (9.2)	12 (8.3)	

*Fisher exact test.

TABLE 3. The association of nephrolithiasis and SNP rs6449213, age, and sex, in a logistic regression model

Variable	Odds ratio (95% confidence interval)	*P*
Age	1.04 (0.99-1.09)	0.108
Sex:		
men (Ref.)	1.00	<0.001
women	0.20 (0.08-0.45)	
SNP rs6449213		
TT (Ref.)	1.00	
CT	2.89 (1.13-7.40)	0.026
CC	1.71 (0.20-15.01)	0.628

TABLE 1. Basic characteristics of nephrolithiasis in symptomatic and asymptomatic participants included in the present study

Characteristics	Symptomatic participants (n = 58)	Asymptomatic participants (n = 87)	*P*
Age; median (interquartile range)	58.5 (18.0)	53.0 (9.0)	0.002*
Women; n (%)	15 (28.8)	61 (75.3)	<0.001†
Body mass index; median (interquartile range)	29.2 (5.2)	26.6 (5.9)	<0.001*
Waist circumference; median (interquartile range)	105.5 (17.0)	87.0 (19.0)	<0.001*

*Mann-Whitney test.
†χ^2 test.

DISCUSSION

This study suggests the association between the SLC2A9 gene and nephrolithiasis, indicating that the SLC2A9 could be the common pathway that links nephrolithiasis and gout (10). Furthermore, the same gene was previously associated with metabolic syndrome (15), thus offering a possible explanation of the link between the 3 disorders. Interestingly, the population in which the gene was originally described has a very high reported prevalence of metabolic syndrome (24), lipid disorders (25), and also increased odds for hypertension (18,26-28), indicating a possible link and the complexity among all these factors and diseases. Also, isolated populations usually have increased odds for nephrolithiasis (29), due to population dynamics and increased prevalence of consanguineous marriages (18,30-33).

The results of our study have to be considered in the light of several serious limitations. The first is the use of heterogeneous and small sample size, especially in comparison with recent genetic studies that have analyzed thousands of samples (34,35). Furthermore, the selection of asymptomatic participants in this study could be the source of bias, which was at least partly overcome by the multivariate analysis method used in the last step of the analysis. The result that suggests that heterozygous individuals have higher odds ratios for developing nephrolithiasis is unusual, especially as the odds ratio of recessive homozygotes was lower than that of heterozygotes. A possible explanation could be that the sample composition could have favored such a result. In turn, this suggests that replication studies will be needed in order to firmly establish the nature of association of this gene with nephrolithiasis. The study also suffers from minimalistic and over-simplistic attempt to explain such a complex phenomenon as the nephrolithiasis by investigating just a set of several variables and ignoring the large number of described confounders. One such confounder is the type of renal excrement, which is often of mixed type, additionally suggesting the complexity of this end-phenotype in humans. The data for this study also did not include sufficient information on metabolic syndrome diagnosing, not allowing us to compare nephrolithiasis status with this disorder and further investigate the link between nephrolithiasis and other metabolic disruptions. Lastly, it might be very interesting to see if the symptomatic individuals were urate stone formers, but this and some other biochemical parameters, were unavailable in this study.

Despite its limitations, this study is, to the best of our knowledge, one of the first studies demonstrating the possible association of SLC2A9 and nephrolithiasis, thus offering a potential for further genetic investigation into the development of nephrolithiasis. It must be noted that the causes for nephrolithiasis are numerous (2) and that SLC2A9 is likely involved in just a fraction of the total variance of the stones formation. To further elucidate the possible linkage of SLC2A9 and nephrolithiasis, it would be needed to repeat this type of study on a much larger sample with detailed clinical history. This would enable the calculation of the percent of nephrolithiasis variance that could be attributed to SLC2A9. Additionally, it might be interesting to perform an intervention study on the patients with known genotypes, aiming to show the changes in the dynamics of urinary excretion associated with mutations of the SLC2A9 gene. Such a study could explain if there are indeed subtle changes in the excretion pattern, which could over time contribute to the development of the nephrolithiasis.

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